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# COMPUTER-ASSISTED SCINTIGRAMS OF THE LIVER AND SPLEEN IN MICE

David Hunter, Captain, USAF, BSC Alan R. Wyant, Staff Sergeant, USAF Robert E. Tatsch, B.S. David Johnson, Technical Sergeant, USAF



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USAF SCHOOL OF AEROSPACE MEDICINE Aerospace Medical Division (AFSC) Brooks Air Force Base, Texas 78235



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#### NOTICES

This final report was submitted by personnel of the Health Physics Branch and Radiation Physics Branch, Radiation Sciences Division, USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, Brooks Air Force Base. Texas, under job order SUPTXHPS.

When U.S. Government drawings, specifications, or other data are used for any purpose other than a definitely related Government procurement operation. the Government thereby incurs no responsibility nor any obligation whatsoever; and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act of 1970 and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources - National Research Council.

This report has been reviewed by the Office of Public Affiars (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

DAVID HUNTER, Captain, USAF, BSC

Project Scientist

ROBERT E. TATSCH. B.S.

and to their

Supervisor

ROY L. DEHART Colonel, USAF, MC

Commander

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## COMPUTER-ASSISTED SCINTIGRAMS OF THE LIVER AND SPLEEN IN MICE

## INTRODUCTION

During the past few years a gamma camera with the pinhole collimator has been used for imaging laboratory animals (1-3). We have, therefore, been interested in a technique that would allow us to observe the sequential in vivo migration of labelled lymphocytes in laboratory mice and to be able to quantitate selected regions of interest from the images. The small size of the mouse results in scintigraphic images too small for accurate interpretation. However, with the use of the l-mm micro-pinhole collimator and the computer, high-quality images are possible for quantitative evaluation.

#### METHODS AND MATERIALS

The mice used for this study were BALB/cJ (Jackson Laboratories, Bar Harbor, Maine), weighing between 18-23 g. The animals were maintained on Purina Laboratory Chow, and tap water ad libitum (cared for by the Veterinary Sciences Division, USAF School of Aerospace Medicine). All scintigrams were made with the mice under anesthesia induced with an intraperitoneal injection of sodium pentobarbital (Nembutal), 25 µg/g.

The lymphocytes (T, B-cell subpopulations) were obtained from the excised spleens of four C57BL/6(H-2b) mice. The In-ll1-oxine complex was added dropwise to the lymphocytes ( $10^7$  cells/ml) and incubated at  $37^{\circ}$ C for 10 minutes with inversion of the cells at 5-minute intervals (4-6). The cells were then washed three times in saline by low-speed centrifugation. Cell viability after labelling, as assessed by NigRosin black dye exclusion, was greater than 92%. The percentage of In-ll1 incorporated in the lymphocytes averaged 89%.

The scintigrams were obtained at 1, 7, and 24 hours after the radiophar-maceutical had been injected into the tail vein of the mouse. The mouse was placed in a supine position at a distance of 5 cm from the face of the pinhole collimator for the 1-hour image and 0.5 cm for both the 7- and 24-hour images. In all cases, 100,000 counts were collected and took approximately 25-35 minutes per view.

The liver and spleen scintigrams were obtained using a Searle Pho-Gamma V Scintillation Camera with a specially made 1-mm micro-pinhole collimator insert and the MDS  ${\sf A}^2$  computer system. The micro-pinhole collimator insert was made of tungsten, measuring 1.75 cm thick and 2.5 cm in diameter.

# RESULTS AND DISCUSSION

This study was undertaken to determine the feasibility of obtaining well-defined scintigraphs of the liver/spleen in the mouse, to assess the possibility of observing the in vivo traffic pattern of In-111 labelled lymphocytes.

The technique has shown that it is possible to obtain well-defined scintigraphs of the mouse liver/spleen. Because this technique does not require sacrificing the mouse to obtain the data, the mouse can be used as its own control and allow sequential imaging to follow the labelled lymphocyte in vivo.

Typical scintigraphs of the liver and spleen are shown in Figure 1, obtained at 1 hour. 7 hours, and 24 hours post injection. The 1-hour image demonstrates

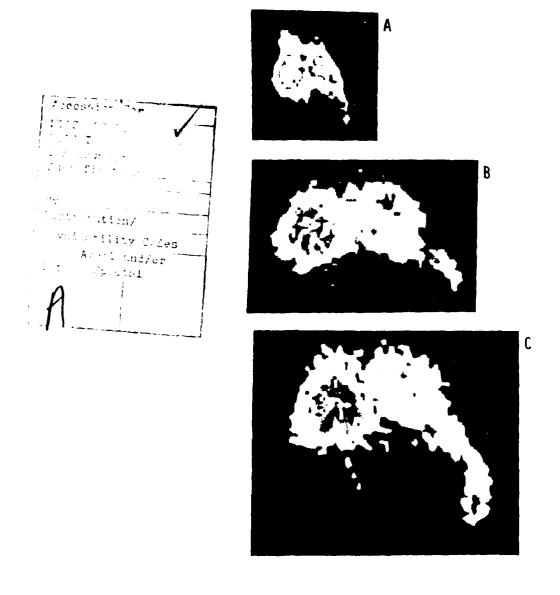


Figure 1. Typical In-111 scintigraphs. Three anterior views were obtained at: (A) 1 hour post injection; (B) 7 hours post injection; and (C) 24 hours post injection.

some activity in the lungs and spleen, with the majority of uptake seen in the liver. The 7-hour image demonstrates total clearance of activity from the lungs with increased uptake in the spleen. At 24 hours the splenic uptake is further increased, which could possibly be related to enhanced cell sequestration of the spleen.

The scintigraphs were useful in following the migration pattern and localization of the labelled lymphocytes. The computer-assisted scintigraphs allow for quantification of selected regions of interest. These areas of interest can be accomplished by the use of either a light pen or cursor techniques from the computer. Both of these techniques are available as part of the quantitative analysis package on the computer.

The use of the labelled lymphocytes with In-111 ( $t_1$  - 2.81 days) allows for delayed sequential scintigraphs. With the relatively high photon flux, delayed scintigraphs can be obtained in about 25 minutes.

#### CONCLUSIONS

Scintigrams shown here demonstrate that high-quality images are possible to obtain from laboratory mice. The use of the computer enhances the scintigram by allowing the investigators to quantitate selected regions of interest in vivo. We would suggest that the micro-pinhole technique be considered by investigators when sequential, quantitative images of small laboratory mice are desired.

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